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DATA EVALUATION RECORD

BAS 500F (PYRACLOSTROBIN)

Study Type: OPPTS 870.3465 [§82-4]; Subchronic Inhalation Study in Rats

Work Assignment No. 3-01-115 (MRID 46638801)

Prepared for
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DATA EVALUATION RECORD

STUDY TYPE: Subchronic Inhalation Toxicity - [rat]; OPPTS 870.3465 [' 82-4]; OECD 413.

PC CODE: 099100 **DP BARCODE:** D322409
TXR#: 0053805

TEST MATERIAL (PURITY): BAS 500F technical (98.7% a.i.)

SYNONYMS: Pyraclostrobin; methyl [2-[[[1-(4-chlorophenyl)-1*H*-pyrazol-3-yl]methyl] phenyl]methoxycarbamate

CITATION: Gamer, A.O., K. Deckardt, S. Burkhardt, *et al.* (2005) BAS 500F – Subacute inhlation study in Wistar rats – 20 aerosol exposures during 4 weeks. Experimental Toxicology and Ecology, BASF Aktiengesellschaft, Ludwigshafen/Rhein, Germany. Laboratory Project Id.#4010494/96073, August 9, 2005. MRID 46638801. Unpublished.

SPONSOR: BASF Corporation, Agricultural Products, P.O. Box 13528, Research Triangle Park, NC

EXECUTIVE SUMMARY: In a subchronic inhalation toxicity study (MRID 46638801), BAS 500F technical (98.7% a.i., Batch # LJ27882/199/b) was dissolved in acetone and administered as an aerosol to 10 Wistar rats/sex/concentration by nose-head only exposure at concentrations of 0 (air), 0 (vehicle control), 0.001, 0.030, or 0.300 mg/L for 6 hours per day, 5 days/week for 28 days (i.e., 20 exposure days).

There were no treatment-related effects on any parameters examined during the FOB or on locomotor activity, ophthalmoscopy, clinical chemistry, or organ weights.

At 0.300 mg/L, abdominal position and moderate labored respiration were observed in one male on Day 21. Additionally at this concentration, the following clinical signs of toxicity were observed [# affected (day of mean onset)]: (i) urine odor in 4 males (Day 29) and in 7 females (Day 24) during daily clinical observations and in one male (Day 28) and 3 females (Day 26) during weekly detailed examinations; (ii) slight visually increased respiration in 10 males (Day 9) and in 10 females (Day 8) during daily clinical observations, and in 8 males (Day 12) and 8 females (Day 14) during weekly detailed examinations (iii) piloerection in 3 females (Day 20) during daily clinical observations and in 3 females (Day 21) during weekly detailed examinations. Additionally at this concentration, four males died prior to scheduled termination

(one each on Days 10, 12, 21, and 22), and three female rats died (one each on Days 7, 11, and 24). Prior to death, these animals exhibited visually increased respiration, urine odor, and piloerection.

At 0.300 mg/L, decreases in body weights of 4-7% (not significant [NS] except on Day 21) and decreases in cumulative body weight gains of 43-141% ($p<0.05$) were noted in the males throughout the study. Food efficiency was decreased ($p<0.05$) at this concentration on Day 7 (-2.6% treated vs 4.0% controls) and on Day 21 (6.5% treated vs 9.9% controls). Additionally on Day 21, food efficiency was decreased in the 0.030 mg/L males (5.8% treated vs 9.9% controls). In both sexes at this concentration, an initial decrease of 11-13% ($p<0.05$) in food consumption was observed on Day 7.

At 0.030 and 0.300 mg/L, diffuse mucosal hyperplasia of the duodenum was observed in 5-7 males (vs 0 controls) and in 5-10 females (vs 1 control). This finding increased with dose in both incidence and severity.

Respiratory effects were observed at 0.030 and 0.300 mg/L, including minimal to slight alveolar histiocytosis in the females (5 treated vs 1 control) and minimal to moderate olfactory atrophy/necrosis in nasal cavities II through IV in the males (2-10) and females (3-9) compared to 0 controls.

Additional effects on the respiratory system at 0.300 mg/L were observed. Lung discoloration was noted at necropsy in 2/10 males and 3/10 females compared to 0/10 animals in each control group. The following findings were observed microscopically (number affected per group out of 10 vs 0 controls, unless otherwise noted): (i) lung congestion in males and females (3); (ii) minimal to moderate hyperplasia of the respiratory epithelium in nasal cavities I through IV in the males (2-10 vs 0-1 controls) and females (7-9); (iii) reactive inflammation in nasal cavity I in the males (2) and females (4); (iv) slight olfactory regeneration/repair in nasal cavity IV in the males (9) and females (8); (vii) minimal to slight respiratory hyperplasia in level III of the larynx in the males (3); and (viii) minimal to slight squamous cell metaplasia in nasal cavity III in the females (4). The numbers of leukocytes were increased by 36% (NS) at this concentration in the males. These data were not available for the females. However, the number and percent of neutrophils were decreased by 38-215% in both sexes. The increased white blood cells (specifically neutrophils) may be an inflammatory response to the treatment-related effects on the respiratory tissues.

The LOAEL is 0.030 mg/L based on hyperplasia in the duodenum, alveolar histiocytosis in the lungs, and olfactory atrophy/necrosis in the nasal tissues. The NOAEL is 0.001 mg/L.

At the request of the Agency, this study was conducted for 28 days, instead of the 90 days required by Guideline OPPTS 870.3465. Aside from the different study duration, this study was conducted in accordance with Guideline OPPTS 870.3465.

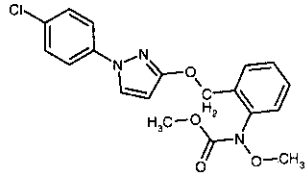
This 28-day study is classified as **acceptable/guideline** and satisfies the guideline requirement (OPPTS 870.3465; OECD 413) for a subchronic inhalation study in the rat.

COMPLIANCE - Signed and dated Data Confidentiality, GLP Compliance, and Quality Assurance statements were provided. A Flagging statement was not provided.

I. MATERIALS AND METHODS:

A. MATERIALS:

1. Test material: BAS 500F

Description:	Red-brown, clear solid melt
Lot/batch #:	LJ27882/199/b
Purity:	98.7% a.i.
Compound stability:	It was stated that the stability of the test article under the storage conditions was confirmed by reanalysis; however, only one purity value was reported. Stability of the test substance in the vehicle was not reported.
CAS # of TGAI:	175013-18-0
Structure:	

2. Vehicle and/or positive control: Acetone

3. Test animals

Species:	Rat
Strain:	Wistar [Crl:WI(Han)]
Age/weight at study initiation:	Approximately 9 weeks old; 225.4-267.5 g males, 158.9-193.9 g females
Source:	Charles River Deutschland GmbH (Sulzfeld, Germany)
Housing:	Individually in type MD III Makrolon wire cages, except during the motor activity measurements which were conducted in polycarbonate cages with wire covers.
Diet:	Milled mouse/rat laboratory diet "GLP" (Provimi Kliba SA, Kaiseraugst, Basel Switzerland), *ad libitum*, except during exposure and motor activity measurements, and overnight (16-20 hours) prior to blood collection and termination
Water:	Tap water, *ad libitum*, except during exposure and motor activity measurements
Environmental conditions:	**Temperature:** 20-24EC **Humidity:** 30-70% **Air changes:** Not reported **Photoperiod:** 12 hrs dark/ 12 hrs light
Acclimation period:	Approximately 2 weeks

B. STUDY DESIGN:

1. In life dates: Start: January 24, 2005 End: February 25, 2005

2. Animal assignment: Animals were randomly assigned, stratified by body weight, to the test groups noted in Table 1. Individual body weights at randomization were within ± 20% of the mean body weight for each sex.

TABLE 1: Study design [a]					
Test group	Target conc. (mg/L) [b]	Analytical conc. (mg/L) [b]	MMAD Φm	GSD	Rats/sex
Control (air)	0	0	---	---	10
Control (vehicle)	0	0	---	---	10
Low (LCT)	0.001	0.00117 ± 0.0003	1.6 ± 0.3	4.0 ± 0.2	10
Mid (MCT)	0.030	0.0304 ± 0.0012	1.9 ± 0.1	2.6 ± 0.1	10
High (HCT)	0.300	0.299 ± 0.015	1.9 ± 0.1	2.5 ± 0.2	10

a Data were obtained from pages 22 and 176 of the study report.

b It was stated that the nominal concentrations could be calculated from the pump rates and air flows during exposure. However, these values were not determined because in aerosol studies they are not comparable and are of no significance to the analytically measured concentrations. Thus, target concentrations are presented.

3. **Concentration selection rationale:** It was stated that the concentrations were requested by the Sponsor, with the 0.300 mg/L concentration expected to cause toxic effects and the 0.001 mg/L concentration expected to be the NOAEL. However, no further concentration-selection rationale was provided.

4. **Test material administration:** The test substance was administered in the vehicle as a liquid aerosol via nose-only inhalation for 6 hours per day, 5 days per week for 4 consecutive weeks (20 total exposures).

5. **Generation of the test atmosphere / chamber description:** Diagrams of the test atmosphere generation system and exposure chamber were included as Figures 1a and 1b on page 71 of the study report. These figures are included in the Appendix of this DER. The test substance was diluted at a ratio of 1 part test substance to 9 parts acetone. For each concentration, test atmospheres were generated as aerosols by supplying the test substance in air (Group 1) or in the vehicle (Groups 2-5) to a two-compartment atomizer (pressure of 0.8-1.2 bars) at a constant rate by means of a metering pump/infusion pump. The aerosol was generated with filtered compressed (approximately 6 bar) air inside the inhalation system at a rate of 1 m^3/h. In the vehicle control group (Group 2), acetone was sprayed at a pump rate equivalent to that of the high concentration group. Supply air was filtered via an activated charcoal filter, conditioned to approximately 50 ± 20% relative humidity and 22 ± 2°C, and supplied at a rate of 5.0-6.0 m^3/h. Actual measurements of the environmental conditions in the exposure chambers resulted in: mean relative humidity ranging between 35.6 and 50.3%; mean temperature of 21.2-22.3°C, and approximately 67 air changes per hour. Although oxygen concentration was not measured, it was stated that the air change rate within the inhalation system was judged to be sufficient to prevent oxygen depletion and that the concentrations of the test substance were not expected to affect the oxygen partial pressure. Time to equilibrium was not reported.

The head-nose exposure system consisted of a cylindrical inhalation chamber made of stainless steel sheeting and cone-shaped outlets and inlets. During exposure, the rats were restrained in glass exposure tubes with their snouts projecting into the inhalation chamber. In order for the animals to acclimate to the exposure system, the rats were treated with supply air under conditions comparable to exposure on the 2 days prior to initiation of the first exposure. Rats were exposed under positive pressure, with an exhaust air flow rate of 5.4 m^3/h.

Test atmosphere concentrations – The concentrations of the inhalation atmospheres were determined by gravimetric analyses and reported as daily means based on two measured samples per concentration per exposure. Results are presented in Table 1 above. The constancy of the concentration of the test atmosphere in each inhalation chamber was continuously monitored using scattered light photometers. Acetone concentration was measured once per exposure in the control and high concentration groups using gas chromatography. It was stated that the stability of the test article under the storage conditions was confirmed by reanalysis; however, only one purity value was reported. Stability of the test substance in the vehicle was not reported.

Particle size determination – Aerosol particle size was determined for each exposure group using cascade impactors. Mass median aerodynamic diameter (MMAD) and geometric standard deviation (GSD) results are presented in Table 1 above. In addition to these parameters, the percent of particles less than 3 µm was measured and ranged from 65.4-71.9%.

6. **Statistics:** The following statistical analyses were performed:

Parameter	Statistical test
Body weight Body weight gain Food consumption Food efficiency	All test groups were compared with the vehicle control group using a two-sided Dunnett's test. The vehicle control group was compared with the air control group using a two-sided Welch t-test
Feces Rearing Fore- and hind-limb grip strength Landing foot splay Motor activity Hematology, except reticulocytes and differential blood count Clinical chemistry Organ weights and terminal body weight	A non-parametric analysis of variance was conducted using a two-sided Kruskal-Wallis test to determine differences among any of the groups. If a difference ($p \leq 0.05$) was detected, then groups exposed to the test material were compared with the vehicle control group using a two-sided Wilcoxon test. The vehicle control group was compared with the air control group using a two-sided Wilcoxon test

Significance was denoted at $p \leq 0.05$ and $p \leq 0.01$. The statistical methods were considered appropriate.

C. METHODS

1. Observations

1a. Cageside observations: All animals were observed for mortality and moribundity twice daily (in the morning and late afternoon) during the week and once daily (in the morning) on weekends and holidays.

1b. Clinical examinations: The clinical condition of each animal was recorded at least three times on exposure days (before, during, and after exposure) and once during pre-flow and on days on which the animals were not exposed. During exposure, only a limited examination was possible due to the animals' location in the exposure tubes. Detailed physical examinations were performed prior to exposure and weekly thereafter. The following CHECKED (X) parameters were examined during handling and in a standard arena:

X	Abnormal behavior during handling	X	Abnormal movements
X	Fur	X	Impairment of gait
X	Skin	X	Lacrimation
X	Posture	X	Palpebral closure
X	Salivation	X	Exophthalmia
X	Respiration	X	Feces (appearance/consistency)
X	Activity/arousal level	X	Urine
X	Tremors	X	Pupil size
X	Convulsions		

1c. Neurological evaluations: A functional observational battery (FOB) and an assessment of motor activity were conducted on all rats on Day 22. Animals were not exposed on the day that the FOB and motor activity tests were conducted. At least 30 minutes prior to testing, the cages were placed in racks in a randomized order, and food and water were withheld. The FOB started with passive observations without disturbing the animals in their home cage, followed by removal from the home cage, and open field observations in a standard arena. Thereafter, sensorimotor tests and reflex tests were conducted. It was stated that a trained technician conducted the FOB, and this technician had performed positive control studies as part of the training. Scoring criteria were provided on pages 424-434 of the study report. The following CHECKED (X) parameters were evaluated during the FOB:

	HOME CAGE OBSERVATIONS		HANDLING OBSERVATIONS		OPEN FIELD OBSERVATIONS
X	Posture	X	Reactivity		Mobility
	Biting	X	Lacrimation / chromodacryorrhea	X	Rearing
X	Convulsions	X	Salivation	X	Arousal/ general activity level
X	Tremors		Piloerection	X	Convulsions
X	Abnormal Movements	X	Fur appearance	X	Tremors
X	Palpebral closure	X	Palpebral closure	X	Abnormal movements
	Feces consistency	X	Respiratory rate	X	Urination / defecation
X	Impairment of gait	X	Red/crusty deposits		Grooming
	SENSORY OBSERVATIONS	X	Mucous membranes /eye /skin color	X	Gait abnormalities / posture
X	Approach response	X	Eye prominence		Gait score
X	Touch response		Muscle tone	X	Bizarre / stereotypic behavior
X	Startle response	X	Pupil size		Backing
X	Pain response	X	Behavior during handling		Time to first step
X	Pupil response				
	Eye blink response		**PHYSIOLOGICAL OBSERVATIONS**		**NEUROMUSCULAR OBSERVATIONS**
	Forelimb extension		Body weight		Hind limb extensor strength
	Hind limb extension		Body temperature	X	Fore limb grip strength
X	Righting reflex			X	Hind limb grip strength
	Olfactory orientation			X	Landing foot splay
X	Visual placing response		**OTHER OBSERVATIONS**		Rotarod performance
X	Pinna reflex	X	Vocalization		

Motor activity was measured in the dark using a Multi-Varimex-System (Columbus Instruments International Corp., Ohio) with four infrared beams per cage. During the measurements, the animals were placed (in randomized order) into polycarbonate cages with absorbent material, and food and water were withheld. The numbers of beam interruptions were counted over 12 intervals, with each interval lasting 5 minutes.

2. **Body weight:** Animals were weighed on Day –7, at the start of the pre-flow acclimation, at the start of the exposure period, once weekly thereafter, and one day prior to scheduled termination. Cumulative body weight gain was reported weekly throughout the study.

3. **Food consumption and food efficiency:** Food consumption was determined for each animal on Day –5 and weekly thereafter throughout the study, and mean daily diet consumption was calculated for each weekly interval as g/animal/day. Food efficiency was calculated from the individual values for each rat by dividing the differences in body weights over an interval by the food consumption during that period and multiplying by 100.

4. **Ophthalmoscopic examination:** Ocular examinations were conducted prior to the initiation of exposure (Day –1 or Day –3) on all animals and on Day 22 for rats in the 0 mg/L (air and vehicle controls) and 0.300 mg/L groups.

5. **Hematology and clinical chemistry:** At scheduled termination, all surviving animals were fasted overnight (16-20 hours) without food (but with *ad libitum* water), anesthetized with isoflurane, and blood was collected from the retro-orbital venous plexus for hematology and clinical chemistry analysis. The CHECKED (X) parameters were examined.

a. Hematology

X	Hematocrit (HCT)*	X	Leukocyte differential count*
X	Hemoglobin (HGB)*	X	Mean corpuscular HGB (MCH)*
X	Leukocyte count (WBC)*	X	Mean corpuscular HGB concentration (MCHC)*
X	Erythrocyte count (RBC)*	X	Mean corpuscular volume (MCV)*
X	Platelet count*	X	Reticulocyte count
	Blood clotting measurements*		
	(Activated partial thromboplastin time)		
	(Clotting time)		
X	(Prothrombin time)		

* Recommended for subchronic inhalation studies based on Guideline 870.3465

b. Clinical chemistry

	ELECTROLYTES		OTHER
X	Calcium	X	Albumin*
X	Chloride	X	Creatinine*
X	Magnesium	X	Urea nitrogen*
X	Phosphorus	X	Total Cholesterol*
X	Potassium*	X	Globulins
X	Sodium*	X	Glucose*
	ENZYMES (more than 2 hepatic enzymes eg., *)	X	Total bilirubin
X	Alkaline phosphatase*	X	Total serum protein (TP)*
	Cholinesterase	X	Triglycerides
	Creatine phosphokinase		Serum protein electrophoresis
	Lactic acid dehydrogenase (LDH)		Albumin/globulin (A/G) ratio
X	Alanine aminotransferase (ALT/also SGPT)*		
X	Aspartate aminotransferase (AST/also SGOT)*		
	Sorbitol dehydrogenase*		
X	Gamma glutamyl transferase (GGT)*		
	Glutamate dehydrogenase		

* Recommended for subchronic inhalation studies based on Guideline 870.3465

6. **Urinalysis**: Urinalysis was not conducted and is not required under Guideline 870.3465.

7. **Sacrifice and pathology:** Following blood collection, all animals were euthanized by exsanguination from the abdominal aorta and vena cava while under Narcoren anesthesia and were subjected to a gross necropsy. The CHECKED (X) tissues were collected from all animals and were fixed in neutral-buffered 4% formaldehyde. Additionally from the liver, samples of the right and left lateral lobes were preserved in Carnoy's solution. These tissues were processed routinely, stained with hematoxylin and eosin, and examined microscopically in the control (air and vehicle) and high concentration groups. Additionally in the intermediate concentration groups, the nasal cavities, lungs, duodenum, and any gross lesions

were examined in both sexes, and the larynx was examined in the males. The (XX) organs, in addition, were weighed.

	DIGESTIVE SYSTEM
	Tongue
X	Salivary glands*
X	Esophagus*
X	Stomach*
X	Duodenum*
X	Jejunum*
X	Ileum*
X	Cecum*
X	Colon*
X	Rectum*
XX	Liver*+
	Gall bladder* (not rat)
	Bile duct* (rat)
X	Pancreas*
	RESPIRATORY
X	Trachea*
XX	Lung*
X	Nasal cavities*
X	Pharynx*
X	Larynx*

	CARDIOVASC./HEMAT.
X	Aorta, thoracic*
XX	Heart*+
X	Bone marrow*
X	Lymph nodes*
XX	Spleen*+
XX	Thymus*+
	UROGENITAL
XX	Kidneys*+
X	Urinary bladder*
XX	Testes*+
XX	Epididymides*+
X	Prostate*
X	Seminal vesicles*
XX	Ovaries*+
XX	Uterus*+
X	Mammary gland (females)*
X	Oviducts
X	Vagina
X	Coagulation glands

	NEUROLOGIC
XX	Brain*+
X	Peripheral nerve (sciatic)*
X	Spinal cord (3 levels)*
X	Pituitary*
X	Eyes (optic nerve)*
	GLANDULAR
XX	Adrenal gland*+
X	Lacrimal gland
X	Parathyroid*
XX	Thyroid*
	OTHER
X	Bone (sternum and/or femur)
X	Skeletal muscle
X	Skin
X	All gross lesions and masses*

* Recommended for subchronic rodent studies based on Guideline 870.3465
\+ Organ weights required

II. RESULTS: Unless otherwise stated, the acetone control group was comparable to the air control group, indicating no effect of the vehicle. Any mention of comparison of treated groups to controls refers to the vehicle control.

A. OBSERVATIONS

1. **Clinical signs of toxicity:** Selected daily clinical observations are presented in Tables 2a and 2b. At 0.300 mg/L, abdominal position and moderate labored respiration were observed in one male on Day 21. Additionally at this concentration, the following clinical signs of toxicity were observed [# affected (day of mean onset)]: (i) urine odor in 4 males (Day 29) and in 7 females (Day 24); (ii) slight visually increased respiration in 10 males (Day 9) and in 10 females (Day 8); and (iii) piloerection in 3 females (Day 20). No other clinical observations could be attributed to treatment. During the detailed clinical observations, similar findings were observed [# affected (day of mean onset)], including: (i) urine odor in one male (Day 28) and in 3 females (Day 26); (ii) slight visually increased respiration in 8 males (Day 12) and in 8 females (Day 14); and (iii) piloerection in 3 females (Day 21). No other clinical observations could be attributed to treatment.

TABLE 2a. Clinical signs of toxicity in male rats during 28 days of treatment with BAS 500 F via inhalation [a]

Observation/parameter	Analytical concentration (mg/L)			
	0	0.001	0.030	0.300
Abdominal position [b]				
Number of animals	0	0	0	1
Number of observations	0	0	0	1
Mean onset (days)	0	0	0	21
Labored respiration, moderate [b]				
Number of animals	0	0	0	1
Number of observations	0	0	0	1
Mean onset (days)	0	0	0	21
Urine odor				
Number of animals	0	0	0	4
Number of observations	0	0	0	10
Mean onset (days)	0	0	0	29
Visually increased respiration, slight				
Number of animals	0	0	0	10
Number of observations	0	0	0	319
Mean onset (days)	0	0	0	9

a Data obtained from Tables IA and IIA on pages 76-77 and 188 in the study report.
b Observed in the same animal (#47).

TABLE 2b. Clinical signs of toxicity in female rats during 28 days of treatment with BAS 500 F via inhalation [a]

Observation/parameter	Analytical concentration (mg/L)			
	0	0.001	0.030	0.300
Urine odor				
Number of animals	0	0	0	7
Number of observations	0	0	0	41
Mean onset (days)	0	0	0	24
Visually increased respiration, slight				
Number of animals	0	0	0	10
Number of observations	0	0	0	336
Mean onset (days)	0	0	0	8
Piloerection				
Number of animals	0	0	0	3
Number of observations	0	0	0	23
Mean onset (days)	0	0	0	20

a Data obtained from Table IA page 78 in the study report.

2. **Mortality:** At 0.300 mg/L, four males died prior to scheduled termination (one each on Days 10, 12, 21, and 22), and three female rats died (one each on Days 7, 11, and 24). These animals exhibited visually increased respiration, urine odor, and piloerection prior to death. All other rats survived until scheduled termination.

3. **Neurological evaluations:** There were no effects of treatment on any parameters examined during the FOB. Locomotor activity was increased by 38% ($p \leq 0.05$) in the 0.030mg/L males compared to vehicle controls during Interval 4. However, because this increase was transient and did not occur at the highest concentration, it was considered unrelated to treatment. Locomotor activity in all other 5-minute intervals and in the overall (12-interval) session was comparable to controls for both sexes and all concentrations. Habituation was unaffected by treatment.

B. BODY WEIGHT AND WEIGHT GAIN: In the 0.300 mg/L males, minor decreases in body weights (↓4-7%) were noted throughout the study, and attained significance on Day 21 (Table 3). Cumulative body weight gains were decreased (↓43-141%; $p \leq 0.05$) in these rats throughout the study. Body weights and body weight gains in the other treated males and in all of the treatment groups in the females were comparable to controls throughout the study.

TABLE 3. Mean (∀SD) body weights and cumulative body weight gains (g) during 28 days of treatment [a]

Day	Analytical concentration (mg/L)			
	0	0.001	0.030	0.300
Males				
0	244.7 ± 9.0	249.8 ± 9.1	244.2 ± 9.1	243.2 ± 12.9
7	250.1 ± 12.3	253.8 ± 11.0	246.1 ± 9.9	241.0 ± 17.3 (↓4)
14	259.3 ± 13.8	264.9 ± 14.5	255.2 ± 10.0	246.4 ± 16.3 (↓5)
21	272.7 ± 14.8	276.2 ± 15.0	263.0 ± 11.3	254.8 ± 18.1* (↓7)
28	280.4 ± 15.0	285.0 ± 16.7	273.0 ± 12.0	265.6 ± 18.3 (↓5)
0-7	5.4 ± 5.3	4.0 ± 7.6	2.0 ± 4.7	-2.2 ± 7.7* (↓141)
0-14	14.7 ± 7.2	15.1 ± 11.8	11.0 ± 4.3	4.6 ± 6.3* (↓69)
0-21	28.0 ± 7.7	26.4 ± 13.7	18.8 ± 5.8	13.0 ± 8.0** (↓54)
0-28	35.7 ± 9.1	35.2 ± 17.7	28.8 ± 7.2	20.4 ± 7.9* (↓43)
Females				
0	174.5 ± 10.2	176.2 ± 8.1	172.9 ± 7.0	172.7 ± 10.7
28	191.2 ± 8.3	187.5 ± 9.3	187.0 ± 8.8	189.0 ± 11.2
0-28	16.7 ± 5.8	11.3 ± 4.4	14.2 ± 5.1	13.5 ± 9.0

a Data obtained from pages 81-82 and 85-86 in the study report; n = 5-10
* Statistically different ($p < 0.05$) from the control.
** Statistically different ($p < 0.01$) from the control.

C. FOOD CONSUMPTION

1. **Food consumption:** At 0.300 mg/L, an initial decrease (↓11-13%; p≤0.05) in food consumption was observed in both sexes on Day 7. There were no other treatment-related differences in food consumption in either sex.

2. **Food efficiency**: In the males, food efficiency was decreased (p≤0.05) at 0.300 mg/L on Day 7 (-2.6% treated vs 4.0% controls) and on Day 21 (6.5% treated vs 9.9% controls). Additionally on Day 21, food efficiency was decreased in the 0.030 mg/L males (5.8% treated vs 9.9% controls). Food efficiency was comparable to controls in the females throughout the study.

D. OPHTHALMOSCOPIC EXAMINATION: There were no treatment-related ocular lesions.

E. BLOOD ANALYSES

1. **Hematology:** At 0.300 mg/L, the numbers of leukocytes were increased by 36% (not significant [NS]) in the males (Table 4). These data were not available for the females. However, the number and percent of neutrophils were increased by 38-215% in both sexes. There were no other treatment-related findings in hematology. A minor decrease (↓4%; p≤0.05) in mean corpuscular hemoglobin concentration was noted in the 0.300 mg/L males. In the vehicle control group, minor differences (p≤0.05) compared to the air control group were noted in the females, including decreased erythrocytes (↓4%), increased mean corpuscular volume and mean corpuscular hemoglobin (↑4% each), and decreased platelets (↓8%). All other findings in the treated groups were comparable to their respective controls.

TABLE 4. Selected mean (∀ SD) hematology findings after 28 days of treatment [a]

Parameter	Analytical concentration (mg/L)			
	0	0.001	0.030	0.300
Males				
Leukocytes (10^9/L)	5.62 ± 1.49	5.71 ± 1.17	6.63 ± 2.03	7.63 ± 3.60 (↑36)
Neutrophils (10^9/L)	0.92 ± 0.29	1.00 ± 0.50	1.11 ± 0.78	2.90 ± 3.70 (↑215)
Neutrophils (%)	16.3 ± 2.0	17.3 ± 5.4	16.1 ± 6.2	31.0 ± 20.0 (↑90)
Females				
Leukocytes (10^9/L)	NA	NA	NA	NA
Neutrophils (10^9/L)	0.56 ± 0.21	0.47 ± 0.11	0.56 ± 0.27	1.13 ± 0.49 (↑102)
Neutrophils (%)	15.7 ± 3.5	13.0 ± 2.9	15.0 ± 4.8	21.6 ± 5.9 (↑38)

a Data obtained from pages 125-130 in the study report. Percent difference from controls, calculated by the reviewers, is included in parentheses. n = 10, except in the 0.300 mg/L group, where n = 6 males and n = 7 females.
NA Not available

2. **Clinical chemistry:** Data for electrolyte and lipoprotein parameters were presented comparing the acetone control groups with the air control groups and comparing the treated groups with the vehicle controls. For enzyme parameters, data were included comparing the acetone controls with the air controls, but were not presented for the treated groups versus the vehicle controls. Based on the data presented, the reviewers determined that there were no treatment-related findings in clinical chemistry. Minor differences ($p \leq 0.05$) were noted in the vehicle control group compared to the air control group, including: (i) decreased alanine aminotransferase (↓20%), aspartate aminotransferase (↓18%), and potassium (↓6%) in the females; (ii) decreased total bilirubin in the males (↓18%); and (iii) increased total protein in the males (↑2%). The only other difference in clinical chemistry was a minor increase of 3% ($p \leq 0.05$) in total protein in the 0.001 mg/L females. All other findings in the treated groups were comparable to their respective controls.

F. URINALYSIS: Not conducted.

G. SACRIFICE AND PATHOLOGY

1. **Organ weight:** There were no treatment-related effects on organ weights. At 0.300 mg/L, absolute and relative (to body) thymus weights were decreased by 27-29% ($p \leq 0.01$) in the females, and relative spleen weight was increased by 33% ($p \leq 0.01$) in the males However, because there were no corresponding microscopic findings in these organs, these differences were considered unrelated to treatment.

   Relative lung weights were increased by 9-11% in the 0.001 and 0.300 mg/L females; however, these increases were not dose-dependent. Additionally in the 0.300 mg/L females, increased ($p \leq 0.05$) relative weights of the kidney (↑6%) and liver (↑12%) were observed; however, these increases were minor and were not corroborated by findings in histopathology. In the male vehicle controls, absolute and relative (to body) lung weights were decreased by 7-8% ($p \leq 0.05$), and relative liver weight was decreased 4% compared to the air control group; however, these decreases were minor and were not considered to be a toxic effect of the vehicle. There were no other significant differences from controls in absolute or relative organ weights in either sex.

2. **Gross pathology:** Lung discoloration was observed in 2/10 males and 3/10 females compared to 0/10 animals in each control group. There were no other macroscopic findings which could be attributed to treatment.

3. **Microscopic pathology:** Treatment-related microscopic findings are included below in Tables 5a (males) and 5 b (females). At 0.030 and 0.300 mg/L, diffuse mucosal hyperplasia of the duodenum was observed in 5-7 males (vs 0 controls) and in 5-10 females (vs 1 control). This finding increased with dose in both incidence and severity. The following remaining microscopic findings were observed in the respiratory system (number affected per group out of 10 vs 0 controls, unless otherwise noted): (i) minimal to slight alveolar histiocytosis in the 0.030 and 0.300 mg/L females (5 vs 1); (ii) lung congestion in the 0.300 mg/L males and females (3); (iii) minimal to moderate hyperplasia of the respiratory epithelium in nasal cavities I through IV at 0.300 mg/L in the males (2-10 vs 0-1 controls)

and females (7-9); (iv) reactive inflammation in nasal cavity I in the 0.300 males (2) and females (4); (v) minimal to moderate olfactory atrophy/necrosis in nasal cavities II through IV in the 0.030 and 0.300 mg/L males (2-10) and females (3-9); (vi) slight olfactory regeneration/repair in nasal cavity IV in the 0.300 mg/L males (9) and females (8); (vii) minimal to slight respiratory hyperplasia in level III of the larynx in the 0.300 mg/L males (3); and (viii) minimal to slight squamous cell metaplasia in nasal cavity III in the 0.300 mg/L females (4). No other microscopic findings could be attributed to treatment.

TABLE 5a. Selected microscopic findings in male rats after 28 days of treatment via inhalation [a]

Microscopic finding		Analytical concentration (mg/L)				
		0 (air)	0 (acetone)	0.001	0.030	0.300
Duodenum						
Diffuse mucosal hyperplasia -	Total	0	0	0	5	7
	>6 – 8 mm^2	0	0	0	5	4
	>8 – 10 mm^2	0	0	0	0	3
Lungs						
Congestion		0	0	0	0	3
Nasal cavity I						
Respiratory hyperplasia -	Total	0	2	0	0	9
	Minimal	0	2	0	0	2
	Slight	0	0	0	0	7
Reactive inflammation		0	0	0	0	2
Nasal cavity II						
Olfactory atrophy/necrosis-	Total	0	0	0	2	10
	Minimal	0	0	0	2	2
	Slight	0	0	0	0	1
	Moderate	0	0	0	0	7
Respiratory hyperplasia -	Total	0	0	0	0	2
	Slight	0	0	0	0	1
	Moderate	0	0	0	0	1
Nasal cavity III						
Olfactory atrophy/necrosis -	Total	0	0	0	5	10
	Minimal	0	0	0	5	0
	Slight	0	0	0	0	6
	Moderate	0	0	0	0	4
Respiratory hyperplasia -	Total	0	0	0	0	10
	Minimal	0	0	0	0	2
	Slight	0	0	0	0	8
Nasal cavity IV						
Olfactory atrophy/necrosis -	Total	0	0	0	9	10
	Minimal	0	0	0	2	1
	Slight	0	0	0	6	5
	Moderate	0	0	0	1	4
Olfactory regeneration/repair -	Total	0	0	0	0	9
	Slight	0	0	0	0	9
Respiratory hyperplasia -	Total	1	0	0	0	8
	Minimal	0	0	0	0	4
	Slight	1	0	0	0	4
Larynx , level III						
Respiratory hyperplasia -	Total	0	0	0	0	3
	Minimal	0	0	0	0	2
	Slight	0	0	0	0	1

a Data obtained from pages 62, 69, and 170-175 in the study report; n = 10.

TABLE 5b. Selected microscopic findings in female rats after 28 days of treatment via inhalation [a]

Microscopic finding		Analytical concentration (mg/L)				
		0 (air)	0 (acetone)	0.001	0.030	0.300
Duodenum						
Diffuse mucosal hyperplasia -	Total	1	1	0	5	10
	>6 – 8 mm^2	0	1	0	5	1
	>8 – 10 mm^2	1	0	0	0	5
	>10 – 12 mm^2	0	0	0	0	3
	>12 mm^2	0	0	0	0	1
Lungs						
Alveolar histiocytosis -	Total	1	1	2	5	5
	Minimal	0	0	1	5	5
	Slight	1	1	1	0	0
Congestion		0	0	0	0	3
Nasal cavity I						
Respiratory hyperplasia -	Total	0	2	0	0	9
	Minimal	0	1	0	0	1
	Slight	0	1	0	0	7
	Moderate	0	0	0	0	1
Reactive inflammation		0	0	0	0	4
Nasal cavity II						
Olfactory atrophy/necrosis -	Total	0	0	0	3	9
	Minimal	0	0	0	3	1
	Slight	0	0	0	0	6
	Moderate	0	0	0	0	2
Respiratory hyperplasia -	Total	0	0	0	0	7
	Minimal	0	0	0	0	2
	Slight	0	0	0	0	5
Nasal cavity III						
Olfactory atrophy/necrosis -	Total	0	0	0	4	9
	Minimal	0	0	0	2	0
	Slight	0	0	0	1	5
	Moderate	0	0	0	1	4
Squamous cell metaplasia -	Total	0	0	0	0	4
	Minimal	0	0	0	0	1
	Slight	0	0	0	0	3
Respiratory hyperplasia -	Total	0	0	0	1	7
	Minimal	0	0	0	0	3
	Slight	0	0	0	1	4
Nasal cavity IV						
Olfactory atrophy/necrosis -	Total	0	0	0	8	9
	Minimal	0	0	0	2	0
	Slight	0	0	0	2	5
	Moderate	0	0	0	4	4
Olfactory regeneration/repair -	Total	0	0	0	1	8
	Minimal	0	0	0	1	1
	Slight	0	0	0	0	7
Respiratory hyperplasia -	Total	0	0	0	1	7
	Minimal	0	0	0	0	5
	Slight	0	0	0	1	2

a Data obtained from pages 62, 69, and 170-175 in the study report; n = 10.

III. DISCUSSION AND CONCLUSIONS

A. INVESTIGATORS= CONCLUSIONS: It was concluded that the LOAEL was 0.030 mg/L based on hyperplasia of the duodenal mucosa and mild to moderate destruction of the olfactory epithelium (characterized by atrophy and/or necrosis, reactive inflammation, and signs of repair/regeneration). Exposure to the test substance resulted in additional direct effects on the respiratory tract at 0.300 mg/L, including hyperplasia of the respiratory epithelium in the nasal and laryngeal tissues and damage to the lungs (as indicated by perivascular inflammatory cell infiltration and alveolar histiocytosis). Aside from effects of the respiratory system, treatment-related findings at 0.300 mg/L included increased mortality and clinical signs of toxicity and decreased body weights, body weight gains, food consumption, and food efficiency.

B. REVIEWER COMMENTS: There were no treatment-related effects on any parameters examined during the FOB or on locomotor activity, ophthalmoscopy, clinical chemistry, or organ weights. Additionally, the few differences noted between the air and vehicle controls (*e.g.*, red blood cell indices) were minor and not considered biologically important.

At 0.300 mg/L, abdominal position and moderate labored respiration were observed in one male on Day 21. Additionally at this concentration, the following clinical signs of toxicity were observed [# affected (day of mean onset)]: (i) urine odor in 4 males (Day 29) and in 7 females (Day 24) during daily clinical observations and in one male (Day 28) and 3 females (Day 26) during weekly detailed examinations; (ii) slight visually increased respiration in 10 males (Day 9) and in 10 females (Day 8) during daily clinical observations and in 8 males (Day 12) and 8 females (Day 14) during weekly detailed examinations (iii) piloerection in 3 females (Day 20) during daily clinical observations and in 3 females (Day 21) during weekly detailed examinations. Additionally at this concentration, four males died prior to scheduled termination (one each on Days 10, 12, 21, and 22), and three female rats died (one each on Days 7, 11, and 24). Prior to death, these animals exhibited visually increased respiration, urine odor, and piloerection.

At 0.300 mg/L, decreases in body weights and cumulative body weight gains were noted in the males throughout the study. Food efficiency was decreased ($p \leq 0.05$) at this concentration on Day 7 and 21. Additionally on Day 21, food efficiency was decreased in the 0.030 mg/L males. In both sexes at this concentration, an initial decrease in food consumption was observed on Day 7.

At 0.030 and 0.300 mg/L, diffuse mucosal hyperplasia of the duodenum was observed in 5-7 males (vs 0 controls) and in 5-10 females (vs 1 control). This finding increased with dose in both incidence and severity.

Respiratory effects were observed at 0.030 and 0.300 mg/L, including minimal to slight alveolar histiocytosis in the females (5 treated vs 1 control) and minimal to moderate olfactory atrophy/necrosis in nasal cavities II through IV in the males (2-10) and females (3-9) compared to 0 controls.

Additional effects on the respiratory system at 0.300 mg/L were observed. Lung discoloration was noted at necropsy in 2/10 males and 3/10 females compared to 0/10 animals in each control group. The following findings were observed microscopically (number affected per group out of 10 vs 0 controls, unless otherwise noted): (i) lung congestion in males and females (3); (ii) minimal to moderate hyperplasia of the respiratory epithelium in nasal cavities I through IV in the males (2-10 vs 0-1 controls) and females (7-9); (iii) reactive inflammation in nasal cavity I in the males (2) and females (4); (iv) slight olfactory regeneration/repair in nasal cavity IV in the males (9) and females (8); (vii) minimal to slight respiratory hyperplasia in level III of the larynx in the males (3); and (viii) minimal to slight squamous cell metaplasia in nasal cavity III in the females (4). The numbers of leukocytes were increased at this concentration in the males. Although these data were not available for the females, the number and percent of neutrophils were decreased by 38-215% in both sexes. The increased white blood cells (specifically neutrophils) may be an inflammatory response to the treatment-related effects on the respiratory tissues.

The LOAEL is 0.030 mg/L based on hyperplasia in the duodenum, alveolar histiocytosis in the lungs, and olfactory atrophy/necrosis in the nasal tissues. The NOAEL is 0.001 mg/L.

At the request of the Agency, this study was conducted for 28 days, instead of the 90 days required by Guideline OPPTS 870.3465. Aside from the different study duration, this study was conducted in accordance with Guideline OPPTS 870.3465.

This 28-day study is classified as **acceptable/guideline** and satisfies the guideline requirement (OPPTS 870.3465; OECD 413) for a subchronic inhalation study in the rat.

C. **STUDY DEFICIENCIES:** The following study deficiencies were noted but do not alter the conclusions of this DER:

- Blood serum enzyme data were not presented for the treated groups versus the vehicle controls. However, there were no treatment-related effects on associated organ weights or histopathology.

- It was stated that the stability of the test article under the storage conditions was confirmed by reanalysis; however, only one purity value was reported. Furthermore, stability of the test substance in the vehicle was not reported. However, the constancy of the concentration of the test atmosphere in each inhalation chamber was continuously monitored.

APPENDIX

Figure 001a: Generator system

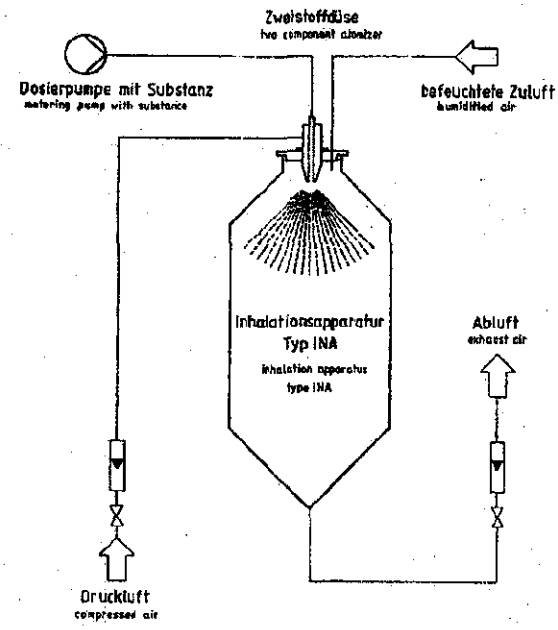


Figure 001b: Exposure system